Original Article

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Comparison of the Effect of Yoghurt Starter Bacteria and Lactobacillous Bulgaricus on Peripheral Blood Mononuclear Cells Activity of Ulcerative Colitis Patients

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ABSTRACT

BACKGROUND AND OBJECTIVE: Ulcerative colitis is an inflammatory bowel disease and involving colon and rectum. Since patients with ulcerative colitis have high levels of pro-inflammatory and anti-inflammatory cytokines, the aim of this study was to compare the effects of starter bacteria of yogurt and lactobacillus boulagaricus on the activity of peripheral blood mononuclear cells in patients with ulcerative colitis.

METHODS: This experimental-laboratory study was performed on 10 ulcerative colitis patients in Dezful, Andimeshk and Shoosh in two experimental groups and a control group. Experimental groups included co-culture of PBMC and intended bacteria in dilutions of 0.1 and 0.01 and at 48 and 72 hours, and control group including PBMC of the patient at 48 and 72 hours. Variables IL-10 and IL-1β) were measured by ELISA.

FINDINGS: There was a significant increase in the secretion of IL1β at dilution of 0.1 and 48 hours by PBMC stimulated with bulgaricus 940.4±249.61 in comparison with the starter 669.12±181.11 (p=0.004) and in 72 hours by bulgaricus 796.3±213.34 in comparison with the starter 464.25±128.41 (p=0.000), In dilution of 0.01 and 48 hours by bulgaricus 747.5±198.54 in comparison with starter 529.25±163.82 (p=0.005) and in 72 hours by bulgaricus 617.4±192.5 in comparison with starter 408.62±134.78 (P=0.004). Also, there was a significant increase in the secretion of cytokines in both dilution and both times between of the experimental groups and control.

CONCLUSION: The results of the study showed that Lactobacillus bulgaricus causes inflammation in comparison with the starter by IL1β secretion. Starter bacteria has a better role in reducing inflammation.

KEY WORDS: *Ulcerative Colitis, Starter, Lactobacillous Bulgaricus.*

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Introduction

Ulcerative colitis is a chronic, recurrent colon disease characterized by mucosal immune deficiency, imbalance in synthesis, secretion of cytokines, and inflammation due to mucosal injury (1). This disease seems to be due to the deficiency of the immune system's tolerance to intestinal antigens, including normal flora and food microbes. The main source of normal flora microbes is dairy products such as yogurt that is prepared using probiotics (3, 2). Studies have shown that various microbial species have different effects on the mucosal immune system, including T regulatory lymphocytes and Effector T cells (4-6). In fact, the differences between strains is shown by the amount of secretion of cytokines in the common culture of probiotic bacteria with peripheral blood mononuclear cells (PBMCs) (7).

Rana et al. showed that patients with ulcerative colitis produce high levels of pro-inflammatory and anti-inflammatory cytokines. This evidence suggests that imbalance between the anti-inflammatory and inflammatory cytokines plays an important role in the pathogenesis of the disease (8). Studies by Javed et al showed that bacteria producing lactic acid can improve inflammatory bowel disease (9). According to Zeuthen et al., probiotic species such as lactic acid producing bacteria produce IL-10 and inhibit T lymphocyte (TH1) and subsequent inflammatory cytokines (10). A study by Donkor and colleagues on probiotics showed that the interaction of probiotics with immune cells and secretion of cytokines caused immune effects on local effective cells. It also causes the differentiation of TH17 and T reg lymphocytes (11). Studies by Elizabet and colleagues showed that the use of probiotics on gastrointestinal patients leads to the production of anti-inflammatory and pro-inflammatory cytokines in regulating the immune responses of these patients and also decreases stress responses (12). Regarding the high prevalence of ulcerative colitis in developed countries and the effects that probiotics can have on the treatment of this disease, this study was conducted to evaluate the effect of yogurt starter bacteria and lactobacillus boulagicus on the activity of peripheral blood mononuclear cells in patients with ulcerative colitis.

Methods

This experimental study was carried out after obtaining permission from Ethics committee of Dezfoul University of Medical Sciences with code: DUR-10.

Samples and groups: Ten patients with ulcerative colitis referring to the clinics of Dezful, Andimeshk and Shoosh districts were selected. During the interview, the information was collected from all individuals including age, duration of the disease, symptoms and diagnosis of the disease, type and dose of consumable drugs. Patients with symptoms such as vomiting and diarrhea (more than twice a day), constipation, presence of blood in the stool, and ulcers in the colon or rectum (by colonoscopy) were included in the study, and if symptoms such as severe intestinal bleeding, cancer, heart and respiratory disease, anemia, age below 15 years and over 60 years old, and patients who were not pleased to participate in this study were excluded. The sample size was obtained 8.81using Fleiss formula for sample size (13), taking into account the test power of 0.8, $\alpha = 0.05$, and the mean change of 5 units, and for more cautious 10 people with Ulcerative Colitis were selected. This number was shared between the experimental and control groups. Sampling was done with informed consent from patients. The study groups consisted of two experimental groups and one control group. The first experimental group consisted of (15, 14) co-culture of mononuclear cells of 10 patients and Lactobacillus boulagicus bacteria in dilutions of 0.1 and 0.01 and at 48 and 72 hours, respectively. The second group also included mononuclear culture of the same 10 patients and starter bacteria in dilutions 0.1 and 0.01 and in two times 48 and 72 hours. Control group also consisted of mononuclear cells of patients without microbial suspension in 48 and 72 hours.

Isolation of mononuclear cells: From each patient, a blood sample of 5 ml was taken and added to the tube containing 2 cc of heparin, then added to the PBS twice as much and homogeneously as possible. Then, 5 ml from the mixture of blood homogeneous and PBS was added to the 3 ml ficoll solution and centrifuged with 2000 rounds for 20 minutes, which, based on the density from the top to the bottom, contained four separate phases including plasma, buffy coat (including

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mononuclear cells), ficoll and red blood cells. After isolating the mononuclear cells, RPMI1640 5 ml was added and centrifugated for 5 minutes. Then, 3 ml CM10 medium (containing 1640 RPMI enriched with calf embryo (FCS 10%) and penicillin antibiotic streptomycin) was added to the precipitate and was placed on shaker for one minute. Finally, the cells were counted under the Invert microscope using a homocytometr slider (16-18).

Buffalo Yogurt: For the preparation of yogurt, the bacteria include Lactobacillus boulagricus (LBY27 from the Kristin Hansen company Denmark) and starter (brand YCX11 from the Kristin Hansen company, Denmark), to a pre-boiled and sterilized formulation with a temperature 45 °C were added separately and placed in an incubator for three to four hours. These steps were repeated until the fifth culture of the bacteria.

Bacterial culture and extraction from yogurt: At this stage, the bacteria were extracted from yogurt, the supernatant formed on yogurt was poured into the falcon tube, and after centrifugation for 5 minutes, the supernatant was removed and added to the MRS broth culture medium. The test tubes were placed at 37 °C for 48 hours in order to grow and settle the bacteria, along with the gas pack, in an anaerobic jar and then in the incubator. After centrifugation, the precipitate was washed three times with PBS and the OD was measured using spectrophotometer (at 598 nm, the absorption was 0.98 and within the solution of 109 bacteria). Then, bacteria were placed under UV light for one night the and killed and placed inside the Ependorf and placed in a freezer at 70 ° C for storage.

Co-culture of bacteria and mononuclear cells: At this stage, dilutions of 0.1 and 0.01 of microbial suspension were prepared (for dilution of 0.01, 180 Landa PBS buffer was added to 20 landa from dilution of 0.1). Two wells of microplate were filled to volume 220 landa (200 landa from cell and 20 landa from microbial suspension with dilution of 0.01 and two other wells with dilution of 0.1). The microplate was then placed in 37 ° C incubator and 5% carbon dioxide for 48 hours. After leaving the incubator, a column of microplate wells was poured into the ependorf, the bacterial name and duration were determined and placed in a freezer at 80 °C; and again, the microplate was placed within the incubator for 72 hours. Then, IL-10 and IL-1β cytokines levels were measured for each of the experimental groups at both dilution and both times, as well as the control group, by the commercial kits made (R & D systems, UK) and Elisa Reader (3200 stat fax of the United States Awareness company). The measurement of kits was based on sandwich ELISA, and the results were obtained in picograms per milliliter. The normal value of IL-10 was 3.9 pg/ml and the normal value of IL-1β was 0.063 pg/ml.

Statistical analysis: The data were compiled using SPSS software version 16 and charts by EXCEL program. First, Kolmogorov Smironov test was used to determine the natural distribution of variables in the research. Then one-way ANOVA was used to examine the difference between groups. In order to show a significant difference between the two experimental groups and without regard to control, the LSD post hoc test and between each of the experimental and control groups were tested by Dunnett T3 post hoc test. Data were presented as Mean ± SD and $p < 0.05$ was considered significant.

Results

A total of 10 people were included in the study, of which 6 were male (60%) and 4 were female (40%). The mean age of the subjects was 13.77±33.8 (17 to 45 years), which was calculated in women (63.6±29.22) and in men (25.28±4.3 years). The results showed a significant increase in IL-10 secretion by boulagaricus in dilutions of 0.1 and 0.01 and 48 and 72 hours, respectively. ($p<0.05$) (Table 1). Also, there was a significant increase in IL-10 secretion by starter in dilutions of 0.1, 0.01 and 48 and 72 hours, compared to control ($p<0.05$) (Table 2).

Regarding the level of IL-1β secretion by boulagaricus, there was a significant increase ($p<0.05$) in the dilution of 0.1, 0.01 and 48 and 72 hours, respectively ($p<0.05$). In addition, there was a significant increase in secretion of these cytokines by starter in dilutions of 0.1, 0.01 and 48 and 72 hours compared to control ($p<0.05$) (Table 4). There was no

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significant difference in the level of IL-10 secretion between the two groups in dilutions of 0.1, 0.01 and 48 and 72 hours ($p<0.05$) (Table 5). However, the comparison between the two groups was related to the level of IL1β secretion. Lactobacillus boulagaricus significantly increased the secretion of these cytokines in dilutions of 0.1, 0.01 and 48 and 72 hours, respectively ($p<0.05$) (Table 6).

Table 1. Indicators of IL-10 secretion by bulgaricus and control in dilutions of 0.1 and 0.01 at 48 and 72 hours

Varibal	Time (hours)	Group	Mean± SD	P-value
IL10-0/1	48	Control	27.84±20.02	0.015*
		Lactobacillous bulgaricus	252.4±193.93	
IL10-0/1	72	Control	41.53±21.05	0.006*
		Lactobacillous bulgaricus	346.49±226.24	
IL10-0/01	48	Control	27.84±20.02	0.004*
		Lactobacillous bulgaricus	381.17±224.29	
IL10-0/01	72	Control	41.53±21.05	0.002*
		Lactobacillous bulgaricus	549.16±328.29	

Table 2. Indices of mean IL10 secretion by starter and control in dilutions of 0.1 and 0.01 at 48 and 72 hours

Varible	Time (hours)	Group	Mean± SD	P-value
IL10-0/1	48	Control	27.84±20.02	0.012*
		Starter bacteria	222.5±133.31	
IL10-0/1	72	Control	41.53±21.05	0.000**
		Starter bacteria	433±140.97	
IL10-0/01	48	Control	27.84±20.02	0.001*
		Starter bacteria	363.87±148.41	
IL10-0/01	72	Control	41.53±21.05	0.000**
		Starter bacteria	527.25±170.61	

Table 3. Indices of IL1β secretion by boulagicus and control in dilutions of 0.1 and 0.01 at 48 and 72 hours

Varible	Time (hours)	Group	Mean± SD	P-value
IL1β -0/1	48	Control	56.3±27.47	0.000**
		Lactobacillous bulgaricus	940.4±249.61	
IL1β -0/1	72	Control	76±25.97	0.000**
		Lactobacillous bulgaricus	496.3±213.34	
IL1β -0/01	48	Control	56.3±27.47	0.000**
		Lactobacillous bulgaricus	745.5±198.54	
IL1β -0/01	72	Control	76±25.97	0.000**
		Lactobacillous bulgaricus	617.4±192.5	

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Table 4. Indices of mean IL1β secretion by starter and control in dilutions of 0.1 and 0.01 at 48 and 72 hours

Varible	Time (hours)	Group	Mean±SD	P-value
IL1β -0/1	48	Control	56.3±27.47	0.000**
		Starter bacteria	669.12±181.11	
IL1β -0/1	72	Control	76±25.97	0.000**
		Starter bacteria	464.25±128.41	
IL1β -0/01	48	Control	56.3±27.47	0.000**
		Starter bacteria	529.25±163.82	
IL1β -0/01	72	Control	76±25.97	0.001*
		Starter bacteria	408.62±134.78	

Table 5. Indicators of IL10 secretion were measured in two experimental groups at dilution of 0.1 and 0.01 at 48 and 72 hours

Varible	Time (hours)	Group	Mean± SD	P-value
IL1β -0/1	48	Lactobacillous bulgaricus	252.4±193.93	0.648
		Starter bacteria	222.5±133.31	
IL1β -0/1	72	Lactobacillous bulgaricus	364.49±226.24	0.252
		Starter bacteria	433±140.97	
IL1β -0/01	48	Lactobacillous bulgaricus	381.17±224.29	0.829
		Starter bacteria	363.87±148.41	
IL1β -0/01	72	Lactobacillous bulgaricus	549.16±328.29	0.833
		Starter bacteria	527.25±170.61	

Table 6. Indices of mean IL1β secretion by two experimental groups in dilutions of 0.1 and 0.01 at 48 and 72 hours

Varible	Time (hours)	Group	Mean± SD	P-value
IL1β -0/1	48	Lactobacillous bulgaricus	940.4±249.61	0.004*
		Starter bacteria	669.12±181.11	
IL1β -0/1	72	Lactobacillous bulgaricus	796.3±213.34	0.000**
		Starter bacteria	464.25±128.41	
IL1β -0/01	48	Lactobacillous bulgaricus	747.5±198.54	0.005*
		Starter bacteria	529.25±163.82	
IL1β -0/01	72	Lactobacillous bulgaricus	617.4±192.5	0.004*
		Starter bacteria	408.62±134.78	

Discussion

The results of this study showed that both experimental groups with dilutions of 0.1, 0.01 at 48 and 72 hours showed a significant increase in the secretion of IL-10 and IL-1β compared to control. In addition, Starter has a better role in reducing inflammation than Lactobacillus boulgaricus due to the lower levels of IL-1β inflammatory cytokine. The results of this study is in contrast with the results of study of Elmadfa et al., indicating that Lactobacillus bulgaricus contributes to secretion of anti-inflammatory cytokines and IL-10 (20). Tomonori et al. showed that Lactobacillus boulgaricus secretes inflammatory cytokines (IL17 and IFN-γ), which is consistent with the results of this study. (21) According to results of Carmen et al., starter resulted in increased levels of IL-10 than IFN-γ and IL-

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10 in comparison with IL-17 in intestinal tissue decreases inflammation intensity (22). The results of this study are consistent with the results of our research. A study by Chapman et al. showed that probiotic mixtures are more effective than single strains against a wide range of inflammatory sites (23). These results are consistent with the findings of this study. Moreno study show that yogurt containing starter bacteria reduces inflammatory cytokines such as IL-12 and 17 (24), which is consistent with the findings of this study. A study by Meyer and colleagues showed that starter yogurt increases inflammatory cytokine (TNFα and IL1β) (25).

This study differs from the results of this study. Hong and colleagues showed that a single strain of Lactobacillus boulgaricus leads to high secretion of inflammatory cytokines (IL1β and TNFα). This amount of secretion decreases for up to 24 hours and then decreases (26). This study is consistent with the findings of our study. An important point in all the findings of this study was that IL-1β secretion was reduced by 72 hours to 48 hours, which could be due to the effect of IL-10, which produced an inhibitory effect on IL1β at 72 hours. In general, the results of this study showed that starter bacteria with anti-inflammatory cytokine secretion (IL10) have a better role in the treatment of inflammation caused by ulcerative colitis than the single strain of Lactobacillus boulgaricus. This result may be due to the effect of Streptococcus thermophilus in a mixture of two bacteria. Therefore, it is suggested that the effect of Streptococcus thermophilus on peripheral blood mononuclear cells in patients with ulcerative colitis should be investigated. Also, the use of anti-IL10 antibody and its effect on the secretion of IL1β in 72 hours is another suggestion.

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